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Mycovirus: Biocontrol agent against *S. sclerotiorum of Rapeseed*

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One of the crucial components of food production is the management of plant diseases. Pesticide usage has horrible impacts on human health as well as significant negative effects on the ecosystem. The widespread use of pesticides has been seen to disrupt the natural populations of many beneficial insects. The employment of biological control methods, which involve the use of numerous helpful microorganisms with the capacity to manage plant diseases, is the best option since environmental contamination has now become a worldwide issue and because chemical control has negative effects. Mycoviruses are viruses that infect fungus, and some of them have the ability to bestow hypovirulence and hence regulate fungal infections. A common ascomycete fungus called Sclerotinia sclerotiorum can harm more than 450 plant species and subspecies. In rapeseed, it produces stem rot, which results in a considerable economic loss every year. S. sclerotiorum has previously yielded a number of hypovirulence-associated mycoviruses, pointing to the possibility that it may harbour different mycoviruses.
Keywords: Biological control, Hypovirulence, Mycovirus, *Sclerotinia sclerotiorum*, RNA-seq, virus transmission.

INTRODUCTION

One of the most important aspects of food production is the control of plant diseases. Control of diseases can be done by using various combined strategies, including breeding suitable disease-resistant cultivars, using crop rotation to avoid the heavy build-up of pathogens, disease-free seeds changed from diseased ones, using flexible planting dates, proper humidity maintained in the field and the use of pesticides to control plant pathogens. Pesticides are the most common and essential practice in controlling plant diseases (Martinelli *et al.*, 2015). The usage of pesticides has detrimental impacts on both human health and our ecosystem. Effective pesticide usage has been shown to disrupt the natural populations of several beneficial insects (Porrini *et al.*, 2003; Rathore and Nollet, 2012). Since that chemical management of plant diseases has a detrimental impact on the environment and that environmental pollution has now become a worldwide issue, the best way to control plant diseases is through the employment of biological control methods. This method includes using various beneficial microorganisms that can potentially control plant diseases (Hyakumachi, 2013). This strategy is best applied in the field by using one beneficial microorganism to control disease-causing microorganisms, and it is environmentally safe and also safe for human health. Ascomycete fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, it is a destructive necrotrophic plant pathogenic fungus that affects more than 450 species and subspecies of plants over 64 genera, and it is to blame for significant losses in agriculture across a wide range of crops throughout the world (Bolton *et al.*, 2006). *S. sclerotiorum* is a bacterium that causes the deadly illness rapeseed stem rot and causes significant losses in China. Each year, this harmful disease reduces the output of rapeseed (Del Río *et al.*, 2007). Rapeseed is an important and one of the most profitable crops in the world. The most important disease of rapeseed is *Sclerotinia* stem rot, also called the white mold of rapeseed (Canola). It is called the most destructive disease of rapeseed. The severity of this disease causes heavy losses to canola production worldwide. Humid weather is favourable for disease development (Kutcher *et al.*, 2011). Mycoviruses are those viruses that infect fungi, and mycoviruses infecting *S. sclerotiorum* are the subject of the present study. Recently, researchers made an outstanding contribution to mycoviruses

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and discovered many novel mycoviruses which a hypovirulence effect on *S. sclerotiorum*, which shows the great potential of mycoviruses to be used as biological control agents (Xie and Jiang, 2014). Mycoviruses are distributed in almost all of the plant pathogenic fungal groups. Transmission of mycoviruses is facilitated by various means, including hyphal transmission, fungal cell wall division facilitated transmission, fungal spores, or insect vectors (*Lycoriella ingenua*). Mycoviruses can cause abnormal growth of fungi and completely stop pathogens' virulence. This way, mycoviruses can help us to control plant diseases which will be a safe and sound method of plant disease control (Kernbauer *et al.*, 2014). Recently, it has been discovered by meta-transcriptomic analysis that these viruses infect 30-80% of fungal species. With the recent advancements in microbiological techniques, especially the development of meta-transcriptomic techniques for discovery, we can no longer understand the functions and nature of mycoviruses (Ghabrial *et al.*, 2015; Liu *et al.*, 2016). Rapeseed (*Brassica napus*) is also called rape or oilseed rape (Schaffner, 1934), and a particular group of cultivars is known as canola. It is a bright-yellow coloured lowering member from the mustard or cabbage family, i.e., *Brassicaceae*. Rapeseed is cultivated worldwide for its oil-rich seeds. It is the world's largest (3rd) source of vegetable oil (George, 2018). Rape, or in Chinese yóucài like other Brassica oil crops, is a very antique crop in China (Needham and Bray, 1984). *B. compestris* var. *chinensis* (bok choy), which has been grown in China since the third century, *B. compestris* var. *glabra*, also known as bai cai, which has been grown for more than 2000 years or even earlier, and *B. compestris* var. *oleifera*, which was grown for seed oil and also used for its tender stems as vegetables (He *et al.*, 2015). From Japan, the common rapeseed *B. napus* was introduced in China in the early 1930s (Liu, 1985). In 1941, the United Kingdom introduced the cultivars with European heritage (Rakow, 2004). The Yangtze basin region of China is one of the three greatest *B. napus* growth locations in the world. The rapeseed planted area during 2016-17 in China was estimated at 7.3 million hectares with 14.55 million tons of forecasted production (USDA, 2017). The entire area under cultivation for the rice-rapeseed systems that were planted in the winter, notably in the Changjiang mid- and low-basin, had been assessed at 7.03 million hectares by the Chinese Ministry of Agriculture. The remaining production is made up of sporadic spring cultivars that were primarily seeded in China's northwest (Clever *et al.*, 2015). Rapeseed mustard is Pakistan's second-most significant oil source after cotton. Rapeseed is grown on 307,000 hectares and produces 233,000 tonnes of oil per year, which accounts for 17% of the local output of edible oil. Rapeseed is grown in sporadic areas throughout Punjab and Sindh.(Pakistan, 2017).

One of the most harmful diseases of rapeseed is stem rot, which is brought on by the fungus S. sclerotiorum. Almost every area where rapeseed is grown has this disease. The need for additional goods forced us to produce dense canopies of rapeseed, which functions as a microclimate for the illness development since a humid environment is most favorable for this disease development, which has resulted in a significant increase in the severity of this disease (Krupinsky *et al.*, 2002). The national rapeseed contemporary industrial technology system's specialists estimated that this illness cost China 8.4 billion RMB in losses in 2017. The fungus overwinters as sclerotia in the soil, stubble, or at the soil surface. Sclerotia are compact masses of hyphae (dormancy structure) that have great potential for viability in the field for up to five years or even more. Sclerotia are dormant in the soil, on diseased plant residues, or sometimes mixed in seeds. The sclerotia can germinate whenever the environmental conditions are favorable (humid) and cause infection. The first visible symptoms of Sclerotinia stem rot occur by the end of the crop's flowering period, and the disease develops late in the season. The appearance of soft watery lesions or areas of very light brown discoloration is the initial symptom, which often appears after two or three weeks of infection. Later stages of infection cause quite visible loss to the crop; even the entire field can give a burnt-like appearance. Sclerotia can also be observed on the surface of the crop (Young *et al.*, 2007).

S. sclerotiorum is a fungus from the Ascomycota division of the kingdom Fungi. It belongs to the family Sclerotiniacea and the genus Sclerotinia. It is one the most destructive plant pathogenic fungi and causes a white mold or stem rot disease. The infections caused by this fungus are called cottony rot, watery soft rot, drop, soft rot, blossom blight and crown rot. The primary characteristic of *S. sclerotiorum* is the ability to produce sclerotia (black resting structures) and white fuzzy mycelial growths on the infected plants. These dormant black structures (sclerotia) produce fruiting bodies in the spring, and from these fruiting bodies, spore production is carried out in a sac. This pathogen can infect a field or a storage facility due to its wide range of hosts. This pathogen can survive on soil, infected tissues and living plants. It can affect plants at any stage, from young seedlings to mature plants. This pathogen quickly spreads from infected to healthy plants in a field. Some commonly infected crops include rapeseed, soybeans, sunflower, green beans, and peanuts. *S. sclerotiorum* spreads in a moist environment. This infection may colonise the entire plant when the environment is wet and can invade its host. This fungus does not like direct sunlight and prefers deeper, shadier environments (Bolton *et al.*, 2006).

Table 1. Important diseases of Rapeseed (*Brassica napus*).

Disease	Name of Disease	Causal agent
Bacterial	Bacterial black rot	*Xanthomonas campestris* pv. *Campestris*
	Bacterial leaf spot	*Xanthomonas campestris* pv. *Armoraciae*
	Bacterial pod rot	*Pseudomonas syringae* pv. *Maculicola*
	Bacterial soft rot	*Pseudomonas marginalis* pv. *Marginalis*
	Scab	*Streptomyces* spp.
	Crown gall	*Agrobacterium tumefaciens*
	Bacterial black rot	*Xanthomonas campestris* pv. *Campestris*
	Bacterial leaf spot	*Xanthomonas campestris* pv. *Armoraciae*
	Bacterial pod rot	*Pseudomonas syringae* pv. *Maculicola*
	Bacterial soft rot	*Pseudomonas marginalis* pv. *Marginalis*
Nematode	Cyst nematode	*Heterodera Cruciferae*
	Lesion nematode	*Pratylenchus* spp.
	Root-knot nematode	*Meloidogyne* spp.
Viral	Crinkle	Turnip crinkle virus (TCV)
	Mosaic	Cauliflower mosaic virus (CaMV)
	Yellows	Beet western yellows virus (BWYV)
Phytoplasma	Aster yellows	Aster yellows phytoplasma
Miscellaneous Diseases and Disorders	Autogenic necrosis	Genetic disorder
	Black speck	Physiological disorder
	Sulfur deficiency	Sulfur deficiency
	Tipburn	Calcium deficiency
Fungal	Alternaria black spot	*Alternaria brassicae*
	Anthracnose	*Colletotrichum gloeosporioides*
	Yellows	*Fusarium oxysporum*
	White rust	*Albugo candida*
	White leaf spots	*Pseudocercosporella capsellae*
	White blight	*Rhizoctonia solani*
	Verticillium wilt	*Verticillium longisporum*
	Southern blight	*Sclerotium rolfsii*
	Root gall smut	*Urocystis brassicae*
	Seed rot, damping-off	*Alternaria* spp.
	Head rot	*Rhizoctonia solani*
	Root rot	*Phytophthora megasperma*
	Ring spot	*Mycosphaerella brassicicola*
	Blackleg	*Phoma lingam*
	Pod rot	*Cladosporium* spp.
	Powdery mildew	*Erysiphe polygoni*
	Leaf spot	*Alternaria alternate*
	Black mold rot	*Rhizopus stolonifer*
	Black root	*Aphanomyces raphani*
	Brown girdling root rot	*Rhizoctonia solani*
	Cercospora leaf spot	*Cercospora brassicicola*
	Clubroot	*Plasmodiophora brassicae*
	Downy mildew	*Peronospora parasitica*
	Fusarium wilt	*Fusarium oxysporum*
	Gray mold	*Botrytis cinerea*
	Sclerotinia stem rot	*Sclerotinia sclerotiorum*

The life cycle of *S. sclerotiorum* is monocyclic because of an absence of secondary inoculums. The fungus will start sclerotium production on or inside the host plant tissues whenever the humid level is favourable for the disease development. The dormant sclerotia of the spring season will start germination, and cup-like, small fruiting bodies called

apothecia will be produced. These fruiting bodies are lined with asci, which have ascospores. After the ascospore releasing, the wind acts as a carrier to the host plants for infection. This fungus can invade almost all plant tissues, including foliage, fruits, roots, flowers and stems. A fluffy white mycelium will begin forming on the diseased area of the plant tissue. *S. sclerotiorum* will again produce sclerotia at the end of the crop growing season until the next growing season; it will remain on the ground surface, in the soil, or on the living or dead plant parts (Bennett *et al.*, 1999*;* Pernezny *et al.*, 2003).

Management of Plant Diseases and Control of Stem Rot Disease: Plant diseases have a direct impact on human civilization by causing severe losses every year to crops. Plant diseases are a global problem for the human population, and the entire population suffers significantly from these diseases. Management and control strategies with novel inventions are crucial for plant diseases. The most important aspect is to have a better and improved quarantine system because a small patch of diseased plant or vegetation can spread the disease over large areas and cause epidemics. All types of plant parts having disease symptoms should be carefully destroyed. On the other hand, cultural practices have great potential to stop or spread disease and improvement in cultural practices can limit the disease spread. Now with the improvement in techniques within the field of agriculture helps us to develop more and more disease-resistant varieties, and by the introduction of disease-resistant varieties, we can better manage the disease problem in the field, but the major problem is that with the development of novel disease-resistant varieties novel types of fungal species also arises; which are a threat for agricultural production. Due to his particular reason, controlling plant diseases using resistant varieties is time-consuming and very difficult (Ainsworth, 1981). One of the most commonly used and famous methods to control plant diseases is chemical control. This method is used in almost all parts of the world. Because chemical fungicide-resistant pathogenic strains are emerging in a field most frequently, it is limiting the efficacy of chemical fungicides, and due to this, the effectiveness of chemical control of plant diseases is often limited. Due to resistance development, we cannot guarantee 100% control of any plant disease with any fungicide available in the market (*Georgopoulos and Skylakakis, 1986)*. Another adverse effect of chemical control is environmental pollution, a severe concern to the world now. Due to these negative effects, the biological control method of plant disease is gaining much more attention and popularity. Using beneficial organisms to control pathogenic microorganisms is a beneficial and environmentally friendly approach (Martinelli *et al.*, 2015). Currently, there is no commercial rapeseed variety with high levels of resistance against stem rot. It is due to the absence of valid screening techniques, difficulties with screening lines being observed under natural conditions and also pathogen variability because it has been observed in the case of *S. sclerotiorum* that this pathogen is reported to be very aggressive and has a wide range of mycelial compatibility grouping; which is considered necessary in the management of diseases caused by the pathogen (Kull *et al.*, 2004). Stem rot disease infection depends upon planting and managing plant population density, and a closed canopy always leads to higher infection rates because of high humidity levels in the field.

Most commonly, all over the world, systemic and non-systemic fungicides are used to control this disease (Khangura *et al.*, 2018). Like many other fungal diseases, the primary focus for controlling stem rot of *S. sclerotiorum* is cultural practices and fungicide treatment. Cultural practices help reduce the sclerotia number present in the soil and create unfavourable conditions for disease development. However, due to the pressure of crop rotation, an increased level of inoculum and cultural practices to control this disease are very limited. Preventive fungicides are mainly used to kill *S. sclerotiorum*, but it is a costly method, and also it is challenging to predict which time is suitable for using these fungicides (Derbyshire and Denton-Giles, 2016). As previously predicted, due to environmental concerns and resistance development against chemical control, we need to focus more and more on biological control methods that can save our environment and improve the overall health condition of the world population (Pretty, 2008). The use of chemical control has now been considered dangerous due to environmental pollution reaching an alarming rate, but before completely stopping pesticides, we need some excellent biological control methods. Using one organism (beneficial) as a biological weapon against another organism (pathogen) is a wonderful and safe technique. This study tries to enhance our knowledge about mycoviral diversity, the presence of novel mycoviruses, and their biocontrol potential in *S. sclerotiorum*, which will help us develop the latest and sustainable biological control strategy against this damaging plant pathogenic fungus.

Mycoviruses, their Evolution and Reported Existence: Viruses that infect and live in fungi. In 1962, the cultivated mushroom *Agaricus bisporus* contained the first mycovirus. The mycovirus-containing mushroom showed less growth and malformed fruiting bodies, resulting in heavy yield losses (Ghabrial *et al.*, 2015). Viruses infecting fungi resemble viruses infecting animals and plants regarding the requirement for a living organism for replication. These viruses have some characteristics which differ them from viruses, such as; they can transmit intercellularly by cell division, they can have an extracellular route as well, as in the case of SsHADV-1 (infection to host is extracellular), mycoviruses do not possess movement protein which is, for instance, the core protein and characteristic of the plant (Son *et al.*, 2015*;* Liu *et al.*, 2016). Infections caused by mycoviruses have been reported in the virulence

(hypovirulence) reduction in a variety of plant pathogenic fungi, and it shows the potential of biological control using mycoviruses (Boland, 2004; Dawe and Nuss, 2013; Ghabrial and Suzuki, 2009). Mycoviruses are believed to exist in all major groups of fungi. Many mycoviruses have been reported to date from old and novel families of viruses. Most of the mycoviruses discovered are dsRNA genomes, but mycoviruses with ssRNA genomes, particularly with +sense ssRNA genomes, are also abundant, covering almost 30% of total reported mycoviruses (*Jiang et al., 2013*). The origin of viruses infecting fungi is still unknown, but there are two different hypotheses about the origin, i.e., some scientist believes that these viruses co-evolved along with their host and other groups of scientist think that these viruses shifted from fungal plant hosts to the specific fungi (Xie and Jiang, 2014; Pearson et al., 2009; Nuss, 2011; Yu et al., 2013*)* . It is still unclear the origin of viruses. According to recent research, the emerging "supergroups" of viruses belong to eukaryotes in a very early stage of life on Earth from an ancestral pool. Koonin (2008) described that RNA viruses were first colonized and then co-evolved with their hosts. This co-evolution theory is compatible with the "ancient co-evolution hypothesis," which postulates that viruses and fungi have long co-evolved (Pearson *et al.,* 2009*;* Varga *et al.,* 2003). The main explanation for the diversity of mycoviruses may be explained by this co-evolutionary hypothesis (Varga *et al.,* 2003*;* Dawe and Nuss, 2001). Also, it has been suggested that plant viruses may have evolved from mycoviruses by adding an extracellular phase to their life cycle as opposed to getting rid of it because they share a movement protein with those viruses. However in many instances, mycoviruses are linked with plant viruses. A good example of this grouping that showed phylogenetic relatedness to the ssRNA genus Potyvirus was CHV1, which commonly revealed that some ssRNA viruses that were thought to induce hypovirulence or debilitation were more closely near to plant viruses (Pearson *et al.,* 2009*;* Fauquet *et al.,* 2005). This gave rise to the hypothesis that these viruses moved from plant hosts to fungal hosts that are plant pathogenic or vice versa. The origins of mycoviruses may not be covered by this idea, but it may explain how they evolved. The discovery of single-stranded circular DNA and negative-strand RNA viruses broadened our awareness of mycoviruses (Ghabrial *et al.,* 2015*;* Yu *et al.,* 2013*;* Yu *et al.,* 2010). A recent discovery of a mycovirus from *Verticillium dahliae* named *Verticillium dahliae* RNA virus 1 (VdRV1), which is closely related to invertebrate viruses, made us understand the relationship and evolution of mycoviruses by supporting heterogenous mycoviral origin hypothesis (Cañizares *et al.,* 2017). Previously, it was thought that transmission might be present between plant and fungal hosts. However, there was no report of such cross-kingdom infection. Recently, cross-kingdom transmission was observed when *Rhizoctonia solani* collected from the field were infected by cucumber mosaic virus (CMV), and this transmission had also been confirmed under laboratory conditions. This discovery changed our thinking about the specificity of plant viruses, as they can also infect fungal hosts and expanded our understanding of the plant-fungus relationship and viral spread (Andika *et al.,* 2017). A similar phenomenon of a plant virus tobacco mosaic virus (TMV) replication in three species of fungi belonging to the Colletotrichum genus was observed, which enhanced our knowledge about host-virus interactions (Mascia and Gallitelli, 2016). The recent discovery of two mycoviruses belonging to families Partitiviridae and Totiviridae had been reported to replicate in tobacco plant cells (protoplasts) without changes in their nucleotide sequences, and this discovery is significant in the understanding of plant-fungus and fungus-plant viral interactions and expands our knowledge about the evolution of mycoviruses (Nerva *et al.,* 2017). Previously, dsRNA viruses forming isometric viral particles or caspidless dsRNA mycoviruses have been reported, but the recent discovery of a novel dsRNA mycovirus forming filamentous viral particles named *Colletrotrichum camelliae* filamentous virus 1 (CcFV-) is reported from *Colletotrichum camelliae*, this virus represented a distinct encapsidating strategy which enhanced our knowledge about the structure of mycoviral particles (Jia *et al.,* 2017).

Model Fungus for Mycoviral Study*:* Chestnut blight fungus (*Cryphonectria parasitica*) belonging to Ascomycota is an essential plant pathogenic fungus and host of various viruses. This fungus is the host of a wide variety of mycoviruses and is therefore considered a model filamentous fungi for virus/host and virus/virus interactions study. This depends upon the development of artificial elimination methods and viral introductions, host genome manipulation, availability of host genome sequence, different strains of host mutants and also largely dependent on molecular tools (*Eusebio-Cope et al.,* 2015; Salaipeth *et al.,* 2014). The history of the first mycovirus identification from *C. parasitica* goes back to 1903 when this fungus was first reported as a hypovirus. Then in 1977, dsRNA elements associated with the transmission of this particular hypovirus for the biological control of chestnut blight were identified; this discovery also led to the development of the term hypovirulence. There is extensive work being conducted on this particular fungus especially using hypovirus CHV-1/EP713; this is the first reported mycovirus in which a reverse genetics system has also been developed; this development allowed us to test the infectivity of mycoviruses following Koch's postulates for better understanding of mycoviruses. With the help of research conducted on CHV-1, we came to know that mycoviruses are considered essential and can probe host functions, and these viruses can be used for the biological control of fungal diseases (Dawe and Nuss, 2013). More and more research has been conducted on this fungus to understand the mycoviruses and recent research conducted by Dong-Xiu Zhang and

Donald L. Nuss reported the engineering of fungal host super-donor strains for *C. parasitica* and these super-donor strains were able to transmit hypoviruses to heteroallelic strains at almost all of the virus-restricting vic loci. With this discovery, we now know that the modulating allorecognition system can engineer pathogenic fungal strains with more efficient transmission of mycoviruses for using them as biological control agents (Zhang and Nuss, 2016).

Metagenomics Study, Presence and Transmission of Mycoviruses: Mycoviruses have been discovered in almost all of the major groups of fungi phylum, including Basidiomycota, Ascomycota, Chytridiomycota, Zygomycota and Deutromycota (Ghabrial *et al.,* 2015). For the discovery of novel and unknown mycoviruses, newly developed techniques like metagenomic are of great importance (*Son et al.,* 2015). Identification of diverse mycoviruses is possible using the metagenomic approach, recently meta-transcriptomic analyses were conducted on five major fungal plant pathogens, including *Colletotrichum truncatum*, *Diaporthe longicolla*, *Macrophomina phaseolina*, *Sclerotinia sclerotiorum* and *Rhizoctonia solani* and these analyses identified 72 partial or complete genome segments including 66 previously undescribed mycoviruses which is an excellent contribution to the identification of mycoviral genomes. The new mycoviruses showed an affinity with 15 distinct lineages in important families of viruses, including Barnaviridae, Chrysoviridae, Benyviridae, Endornaviridae, Hypoviridae, Fusariviridae, Mononegavirales, Ophioviridae, Narnaviridae, Ourmiavirus, Tombusviridae, Partitiviridae, Totiviridae, Virgaviridae and Tymoviridae. More than 50% of the viral sequences were predicted to be members of the family Narnaviridae. It showed the importance of meta-transcriptomic analysis in the field of mycovirology (Marzano *et al.,* 2016). Metagenomics is crucial in mycovirus discovery, and this technique is helpful in many ways. A wide range of partitiviruses associated with mycorrhizal Ceratobasidium fungus has been discovered using a combination of dsRNA enrichment and metagenomics using shotgun high-throughput sequencing technique from Australia over 2 years consecutively. A total of 21 partial and some near-complete sequences from 16 different alpha and betapartitivituses were detected from this metagenomics analysis from 2 fungal isolates, and it showed the co-infection in a fungus by multiviruses (Ong *et al.,* 2017). Another study conducted using high-throughput sequencing on powdery mildew-causing pathogen Erysiphe necator from a grapevine. This study showed the presence of 8 mycoviruses related sequences from E. necator, including five from Partitiviridae named Erysiphe necator partitivirus 1-3 (EnPV 1-3) and three from Narnaviridae named Erysiphe necator mitovirus 1-3 (EnMV 1-3) (Pandey *et al.,* 2018). Another recent example of high-throughput sequencing used for the discovery of a novel dsRNA mycovirus, which is the first report of a mycovirus from an isolate of *Neofusicoccum leteum* collected from grapevines, and this dsRNA mycovirus had been named as *Neofusicoccum luteum* fusarivirus 1 (NIFV1) and placed in the recently proposed novel family Fusarivirade. This discovery showed the potential for mycovirus discovery using a metagenomics approach. With the help of metagenomics, we can discover more novel viruses from a wide range of fungal hosts (Marais *et al.,* 2018). Recently, 84 strains of *S. sclerotiorum* collected from Australia were used to determine the diversity of mycoviruses using metatranscriptomic analysis. This study revealed the presence of partial or complete genomes of 57 mycoviruses, with 34 being novel viruses. These 47 mycoviruses belong to 10 different lineages, including Endornaviridae, Genomoviridae, Hypoviridae, Mononegavirales, Narnaviridae, Partitiviridae, Ourmiaviruses, Tombusviridae, Totiviridae, Tymovirales, and some viruses from non-classified families were also detected. This study enhances our knowledge about the diversity of mycoviruses worldwide and also elaborates on the importance of meta-transcriptomic analysis in identifying viral genomes (Mu *et al.,* 2018). Many mycoviruses are being reported from a wide range of fungal hosts, such as reporting of *Rhizoctonia solani* flexivirus 1 (RsFV1), which suggested the plasticity of genomes within the order Tymovirales; not only this report of a novel virus but it can also infect the host fungus and cause it to become avirulent; which shows the potential for mycoviruses to be used as a biological control method for prevention of diseases (Bartholomäus *et al.,* 2017). Mycovirus discovery from a strain of *Trichoderma atroviridae* named *Trichoderma atroviridae* mycovirus 1 (TaMV1) showed the diversity of mycoviruses by infecting a wide range of fungal members, including Trichoderma (Lee *et al.,* 2017). Previously, dsRNA viruses forming isometric viral particles or caspidless dsRNA mycoviruses have been reported, but the recent discovery of a novel dsRNA mycovirus forming filamentous viral particles named *Colletrotrichum camelliae* filamentous virus 1 (CcFV-) is reported from Colletotrichum camelliae, this virus represented a distinct encapsidating strategy which enhanced our knowledge about the structure of mycoviral particles (Jia *et al.,* 2017). Mycoviruses, in most cases, do not show any symptoms on their host after infection. With the help of spores' natural dissemination, these viruses are vertically transmitted; in other cases, these can be transmitted horizontally by intracellular hyphal anastomosis.

One of the critical reasons for mycoviruse's presence in all major phyla of fungi is that they do not transmit vertically and horizontally together. According to recent estimates, about 30-80% of fungal species are infected by mycoviruses (Ghabrial and Suzuki, 2009; Nuss, 2005). Mycoviruses most frequently spread by means of horizontal transmission, which is done through hypha fusion. With a few notable exceptions, most mycoviruses can only transmit between genetically compatible strains. For instance, the hypovirulence-associated mycovirus SsMYRV4 was discovered to suppress

the host's non-self-recognition system by facilitating the horizontal transmission of heterologous viruses (Saupe, 2000; Wu *et al.*, 2017). Additionally, SsHADV1 and other viruses SsPV1 showed potential to be transmitted non-sexually (Yu *et al.*, 2013). FgV1-DK21 transfer from Fusarium boothii to *Cryphonectria utilising* protoplast fusion is one of the first and best examples of mycovirus transmission from one isolate to another. The recipient strain had slower growth rates and less coloration. Also, it reduced virulence, which showed the potential of this virus to be used as biological control agent (Lee *et al.*, 2011). Another method of transmission for mycovirus is vertical transmission, that is, through asexual or sexual spores; the best example of vertical transmission is CHV1 (Cryphonectria hypovirus 1), with exceptionally high efficiency of transmission through conidia (Melzer *et al.*, 1997).

Mycovirus transmission by sexual spores is sporadic, and not many viruses are discovered to be transmitted by sexual spores. Only one dsRNA mycovirus discovered from rice blast fungus *Magnaporthe grisea* has only 10% transmission efficiency utilizing sexual spores. Mainly three crosses were made between dsRNA-containing and dsRNA-free strains, including sib-mating, parental and backcrossing and 11 out of 105 ascospore progenies contained the virus (Chun and Lee, 1997). Recently, a mycophagous insect, *Lycoriella ingenua*, has been discovered to be infected by SsHADV-1 and therefore act as a vector for mycovirus transmission and the larvae of this insect after feeding on SsHADV-1 infected fungi; contained the virus and then the virus retained and replicated as well into all the stages of insect development including larvae, pupae, adults and also in the eggs. The viral transmission was confirmed by injecting the larvae with SsHADV-1 viral particles, and the virus was transmitted to the insect's offspring when they were allowed to feed on nonhost fungus. SsHADV-1 infected insects were also captured from the rapeseed field, which showed the potential of this virus transmission using insect vectors, particularly *Lycoriella ingenua*. The virus-infected female insects were observed to produce more eggs compared to uninfected adults, which suggests that this insect can transmit the virus to more fungi and help us control the disease (Liu *et al.*, 2016). It has been suspected that viral infection transmission between fungal and plant hosts may occur. Many mycoviruses have been reported to be closely related to plant viruses. However, this phenomenon has never been discovered in nature, but with the recent discovery of a naturally occurring infection of *Rhizoctonia solani* by a plant virus named cucumber mosaic virus (CMV), this phenomenon has been confirmed (Andika *et al.*, 2017).

Mycoviruses in Sclerotinia sclerotiorum: Hypovirulence-associated mycoviruses with potential hypovirulence association can help us develop mycoviral biological control agents to help reduce losses by *S. sclerotiorum* to rapeseed crops. *S. sclerotiorum* has been recognized to contain a wide range of viruses, and some confer hypovirulence (Xie and Jiang, 2014; Yu *et al.*, 2013). Many *S. sclerotiorum* strains containing mycoviruses have recently been reported with viruses, including hypoviruses and Alphaflexiviridae, Endornaviridae, Gemonoviridae, Myconoviridae, Narnaviridae, Reoviridae, Megabirnaviridae, Narnaviridae and the newly proposed families such as from Fusariviridae and Deltaflexiviridae (Marzano *et al.*, 2016; Wu *et al.*, 2017; Xie *et al.*, 2011; Khalifa and Pearson, 2014; Xu *et al.*, 2015; Wang *et al.*, 2015) . There is various evidence of a single strain of *S. sclerotiorum* infected by multiple viruses, such as strain Ep-1PN, had been reported to be a hypovirulent strain with 3 dsRNA elements L, M and S segment (M segment identified as Sclerotinia sclerotiorum debilitation associated RNA virus and L segment identified as *Sclerotinia sclerotiorum* RNA virus L) (Jiang *et al.*, 1988; Liu *et al.*, 2009). Another evidence of a single strain of *S. sclerotiorum* co-infected by a bi-segmented dsRNA virus (SsBRV2/AH16) and a nonsegmented +ssRNA virus (SsMV4/AH16) has been reported, which broadened our knowledge about co-infection of different mycoviruses in a single strain of fungi (Ran *et al.*, 2016). Another example of co-infection of a single strain with different viruses includes the discovery of three mitoviruses identified from an isolate of *Sclerotinia sclerotiorum*, as well as horizontal and vertical transmission of these newly reported mycoviruses, which were studied. This study showed that mitovirus infection significantly reduced host growth and virulence. A new genus of mycovirus has been proposed, "Gamahypovirus", new genus had been proposed (Gamahypovirus) in the family Hypoviridae by the reporting of a novel virus identified from *S. sclerotiorum* named *Sclerotinia sclerotiorum* hypovirus 2. The role of recombination in Hypoviridae evolution was also clarified (Khalifa and Pearson, 2014). Two new +ssRNA viruses co-infected hypovirulent strain of *Sclerotinia sclerotioruim* were reported with the determination of their full-length genomes. A novel SsDRV/SX247 was reported from Sclerodarnavirus, and SsHV2/SX247 was reported with close relation to genus Alphahypovirus and in the case of SsHV2/SX247 is a member of the newly proposed genus Gammahypovirus. It has been demonstrated that purified particles of a DNA mycovirus (*Sclerotinia sclerotiorum* hypovirulence-associated DNA virus 1 (SsHADV-1), are highly infectious when these are applied extracellularly to *S. sclerotiorum*. These findings significantly contribute to understanding mycoviral interactions with their hosts and explain biological control using mycoviruses (Yu *et al.*, 2013). The understanding of mycoviruses occurring in a natural environment had been significantly enhanced with the discovery of *Sclerotinia sclerotiorum* hypovirulence associated DNA virus 1 (SsHADV1) in the samples of benthic and bank river sediments of two urban rivers in the city of Christchurch (New Zealand). It was the first report of SsHADV-1 outside China and in environmental samples. A recently characterized

dsRNA mycovirus named *Sclerotinia sclerotiorum* megabirnavirus 1 (SsMBV1) from the hypovirulent stain of *Sclerotinia sclerotiorum* and reported horizontal gene transfer from ssRNA virus to dsRNA virus (Wang *et al.,* 2015). High throughput sequencing helped us discover mycoviruses in regions where mycoviruses were not previously discovered from a specific plant pathogenic fungus. The best example is the discovery of a wide range of mycoviruses from *S. sclerotiorum* from Australia. Previously no mycovirus were reported from this region, particularly from *S. sclerotiorum*. Recently, a study was conducted on 84 isolates collected from Australia. It was discovered after RNA-sequencing that a wide range of mycoviruses infected these isolates, and a total of 285 contigs representing partial or complete genomes of 57 mycoviruses were obtained, and out of these sequences, 34 novel viruses were obtained as well. It enhanced our overall knowledge about viral diversity in different regions of the world (Mu *et al.*, 2017).

Importance and Limitations of Biological Control Using Mycoviruses: Fungi are the primary cause of diseases in the field, causing heavy losses in terms of crop yields and also causing significant disturbance to our food security. Most of the fungal pathogen's resistance development against the host plant's natural protection mechanism is very quick and is the reason for rapid disease development in the host plants. As previously discussed, the problem with using fungicides is the rapid development of resistance in fungal pathogens against chemicals and environmental contamination risk. So, considering all the factors, an alternate and sustainable plant disease management strategy is needed. Mycoviruses can be an alternative because there are fewer chances of resistance by fungal hosts against mycoviruses as well as these are environmentally safe and an effective method for disease control (Xie and Jiang, 2014; Marzano *et al.,* 2016) . Mycoviruses can have a significant impact on the productivity and health of plants; for example, in the case of CHV1 and SsHADV-1, these viruses significantly reduced the fungal growth and dramatically reduced the infection, which led to thinking and discovery of more and more novel viruses to be used as bio-control agents (Yu *et al.*, 2010; Xie *et al.*, 2006; Anagnostakis, 1982). Using mycoviruses to control plant diseases has old history back to the 1950s when a hypovirus were discovered for the biological control of chestnut blight from a hypovirulent strain of *C. parasitica* due to the observation of an Italian phycologist Biraghi observed strange phenomena when several trees infected by *C. parasitica* were not killed (BIRAGHI, 1953). Biological control of chestnut blight with the help of hypovirus is dependent on the natural spread of the virus and the interaction between the virus, pathogen and tree, as well as environmental factors (Milgroom and Cortesi, 2004). An excellent example of the recent development in mycovirus research for biological control, particularly on *C. parasitica*, is the engineering of a super donor strain of *C. parasitica* that can donate the mycovirus to virus-free fungal strains is a significant step forward in the understanding and use of mycoviruses for biological control of fungal diseases (Zhang and Nuss, 2016). Another good example is the potential of SsHADV-1 to control stem rot of rapeseed; SsHADV-1 has strong infectivity and can transmit between incompatible vegetative strains of *S. sclerotiorum* by hyphal anastomosis (Yu *et al.*, 2010). In addition, the purified viral particles of SsHADV-1 can easily infect the healthy hyphae of *S. sclerotiorum* (*Yu et al.,* 2013). When it was attempted to spread the hyphal fragments of the SsHADV-1 virus-infected strain over the surface of rapeseed leaves, the lesion expansion was suppressed, and the vegetative-incompatible strain was found to be infected by SsHADV-1. It shows the great potential of mycoviruses to be used as biological control agents (Xie and Jiang, 2014). With advances in molecular research and the introduction of meta-transcriptomic analysis (RNA-sequencing) techniques to identify viral genomes, our knowledge and understanding of mycoviruses have been broadened (Son *et al.,* 2015). Mycoviruses are essential to control plant diseases, but the efficiency of mycoviruses for biological control of plant diseases can be limited (*Pearson et al.,* 2009). The most critical limitation in biocontrol is the complicated vegetative incompatibility between host isolates which limits the viral transmission from infected to healthy strains of the fungal pathogen (Pearson *et al.,* 2009). Another important limiting factor is the fitness field in case of virus-infected strains, which stops the natural spread of the virus among different fields, and the viral spread is also limited by fungal pathogens and infected plant interactions as well as environmental conditions (Pearson *et al.,* 2009).

Conclusion and future aspects: A diverse range of mycoviruses has ssRNA positive-strand, negative-strand, dsRNA and ssDNA genomes, respectively. This Meta transcriptomic analysis shows that mycoviruses are naturally occurring and infecting plant pathogenic fungi, especially in the case of *S. sclerotiorum*. Metagenomics analysis has excellent importance in mycovirus discovery, and recently many novel mycoviruses have been discovered using the metagenomics approach. Meta-transcriptomic analysis showed that an increasing number of circular DNA viruses having single strands had been reported from fungi. Metagenomics involves using various approaches, such as using enriched samples for viruses using biological, chemical, or physical methods. To date, there are many mitoviruses discovered from *S. sclerotiorum*, including *Sclerotinia sclerotiorum* mitovirus (1, 2, 3, 4, 5, 6 and 7). Metagenomics study also found many already reported mitoviruses, along with novel mitoviruses from *S. sclerotiorum* strains from two different countries, which showed that mitoviruses are the most frequently found mycoviruses within *S. sclerotiorum*. Identification of a diverse range of mycoviruses in plant pathogenic fungi has a wide range of implementation, including managing fungal diseases and understanding the

viruses and their nature. There is great potential in using mycoviruses as biocontrol agents in the field, and by infection of pathogenic fungi with virulence-altering mycovirus, we can reduce the disease incidence and severity. This diversity of mycoviruses within one plant pathogenic fungus shows that mycoviruses are widespread and can multiply quickly and spread over large areas to infect plant pathogenic fungi. More and more information regarding mycoviruses can increase our knowledge and clarify fungi's interactions with these viruses. This metagenomics study enhanced our knowledge about the discovery and diversity of mycoviruses from *S. sclerotiorum*, but the further characterization of these mycoviruses is warranted for the proper placement of these viruses.

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